SIGNIFICANCE OF THE COLON-AEROGENES GROUP IN ICE CREAM

Thesis for the Degree of M. S.

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THESIS

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Introduction.

A review of the industrial development of this country shows plainly that the surviving and prosperous industries are those which have tended to build larger and larger units. This consolidation generally results in a better product for the consumer. This has been particularly noticeable with respect to the ice cream industry. Gradually but steadily the small plants have been assimilated by the larger organizations. The result has been a noteworthy increase in the sanitary quality of the products offered for sale.

Along with this industrial growth there has been a steady growth of those agencies designed to protect the public from insanitary products. The new facts constantly being discovered are broadening the field of the public health worker. The work of the past few years has indicated a considerable amount of interest in the sanitation of frozen milk products. As the new facts have been made available, the vigilance of the public health authorities has increased correspondingly. New legislation is constantly being enacted better to control the sanitary quality of dairy products.

At the present time the facts available to the public health officer are meager and of those standards

which exist, many have not been found acceptable. The confusion and misunderstanding which has resulted is only a natural phase in the development of suitable criteria.

The following work was undertaken with the express desire of obtaining further information upon the significance of the colon-aerogenes group in ice cream. In connection with this problem several questions present themselves. Does the presence of this group in retail ice cream indicate inefficient pasteurization or contamination after pasteurization? What is the possibility of members of this group surviving the pasteurization temperatures now in use? Is the health officer justified in condemning ice cream which contains members of the colon-aerogenes group? It is hoped that the material submitted may be of some value in answering the above questions.

Historical.

Since the discovery by Escherich in 1886 of Escherichia coli, this organism and related species have been a constant center of discussion and investigation. As soon as workers had established the fact that Escherichia coli was a constant inhabitant of the intestinal tract, its presence in water supplies was regarded as an index of fecal pollution. Numerous methods for examination were advanced with the result that confirmatory work was scarce and no standard form of procedure was adopted.

After extensive work onstream pollution in 1901, Jordan (1) concluded that whenever extensive mortality occured among the colon bacteria a similar mortality could be assumed to have occured among the typhoid bacteria. Savage (2) in 1902 stated that although a knowledge of the number of Escherichia coli present is essential, arbitrary standards of allowable numbers are of little value. In 1903 Horton (3) studied ground waters and concluded that the presence of Escherichia coli in drilled wells is generally sufficient evidence to condemn that source. Stoughton (4) in 1905 questioned the value of the presumptive test and presented data to show the unreliability of this method of procedure. In the same year the Committee on WaterAnalysis of the American Public Health Association published their report. These findings and the revised standards following have provided a standard procedure for the bacteriological examination of water supplies. Detailed qualitative and quantitative procedure for the determination

of Escherichia coli and related species is an important feature of this report. The sum of the work done on Escherichia coli in water supplies tends to show: First, that this organism multiplies very little, if at all, in natural water supplies; next, that a quantitative determination of members of this group is of more importance than the qualitative test; and that the source of the members of this group is of great importance in determining the potability of a suspected water supply.

As soon as the colon test for water supplies had become firmly established, investigators turned their attention to milk. One of the earlier papers in this field was published in 1908 by Bergey and Deehan (5). The authors isolated fifty strains of the colon-aerogenes group from milk. The early work of this nature was concerned primarily with the isolation and identification of members of this group.

Not until the pasteurization of milk became a common practise did bacteriologists attempt to find a reliable method for determining the efficiency of the pasteurization process. Numerous studies were made upon the flora of pasteurized milk. In 1910 Shippen (6) isolated thirty-one strains of the colon-aerogenes group from pasteurized milk. He found that eleven of these strains possessed a thermal death point above 60° C. for fifteen minutes. Three of the strains survived 62° C. for thirty minutes and three others 63° C. for thirty minutes. The author concluded, "that the thermal death point of a given species varies with the different strains of that species. Certain strains of

Escherichia coli are not killed by the temperatures commonly used in pasteurization. The presence of Escherichia coli in pasteurized milk cannot be taken as an index of improper pasteurization or subsequent contamination." In the same year Ayers and Johnson (7) confirmed by an extensive study the variation of the thermal death point of members of this group. Two hundred and seventy-four cultures were isolated, the majority being obtained from cow feces. Their method was to inoculate tubes of sterile litmus milk with four drops of a broth culture of the strain to be studied. The tubes were then heated in a constant temperature water bath at various temperatures for thirty minutes. The tubes were cooled immediately and incubated at 37° C. The results obtained indicated that many strains have a thermal death point close to 62.8° C. (145° F.). They concluded that this fact complicates the colon test, if used as a test for efficiency of pasteurization, and further, that Escherichia coli has a low majority thermal death point and the survival of some strains is due to a few resistant cells. The finding of large numbers of Escherichia coli immediately after pasteurization might be interpreted as being due to inefficient heating or a heavy recontamination. Another paper by the same authors (8) published in 1924 showed that a pasteurization temperature must be based upon the absolute thermal death point as determined in the laboratory. They concluded that large scale efficiency tests were not only unnecessary, but were apt to be misleading. Jenkins (9) in 1926 studied the flora of pasteurized milk and determined the

colon organism content by means of bile salt lactose peptone water. The author stated that an effectively pasteurized milk should not contain lactose fermenting bacilli in one cubic centimeter and that the Escherichia coli is a valuable index of the efficiency of the pasteurizing process.

Sixteen milk plants were studied by Swenarton (10) in 1927, in an effort to correlate plant performance with the Escherichia coli content. He used the term Escherichia coli to indicate all members of the group of non-spore forming aerobic bacilli which ferment lactose with the production of gas. The work was summarized as follows: "The coli content of pasteurized milk was found to vary considerably. Control charts from milk plants whose milk was high in coli content showed improper heating or irregularity of procedure. The charts indicated a definite correlation between coli content and plant procedure. A test for Escherchia coli can be used to check up on plant performance." A standard was proposed for the maximum Escherichia coli of pasteurized milk.

A paper by Rudge (11) in 1927 pointed out the difference between the colon count in water and in milk. The author stated that the significance of the colon organism when found in milk is in no way as great as when found in water. This fact is attributed to the conditions peculiar to the gathering of milk which cannot be entirely eliminated.

The scientific literature contains numerous papers concerning the bacteriology of ice cream, but very few of these contain special reference to the colon-aerogenes group of organisms. Buchan (12) in 1910 made a complete bacterio-

logical study of fifty small ice cream plants in England. Out of sixty-six cultures isolated and identified forty-seven belonged to the colon-aerogenes group. Isolation was accomplished by the use of bile salt lactose agar. The author presented a bacteriological standard for ice cream which limited members of the colon-aerogenes group to 0.1 cc. of ice cream as measured by the production of acid and gas in bile salt glucose broth. One of the most complete papers on this subject was recently published by Weinzirl and Harris (18). Three methods for determining the sanitary quality of ice cream were studied and compared. These were the total count, the colon-aerogenes count and the anerobic spore test. The method used for determining the colon-aerogenes count was as follows: The sample to be tested was diluted 1 to 10, 1 to 100, and 1 to 1,000,000. Lactose bouillon enrichment tubes were inoculated with 0.1 cc. portion of each dilution and all tubes showing more than 10 per cent gas were plated on Endo's agar. From these plates typical colonies were fished and identified according to the Standard Methods for Water Analysis as furnished by the American Public Health Association. The authors concluded that the colon-aerogenes count is of value in controlling the efficiency of pasteurization and that it also indicates insanitary conditions. The fact that it does not distinguish between initial contamination and subsequent multiplication was cited as a distinct disadvantage.

This brief summary indicates the need of further contributions to this field of research. The greatest need

seems to be for a standard method of interpreting the significance of members of this group when found in ice cream. However, it is evident that this problem applied to ice cream presents difficulties not present when applied to milk. The numerous differences between these two dairy products demands more investigation before definite conclusions may be reached. Very little work has been done to indicate those factors which may or may not affect the viability of members of this group. This phase alone should be of sufficient value to attract further investigation.

Procedure and Results

At the outset of the following study it was necessary to adopt an acceptable method of pasteurization. Owing to the large number of cultures used it was necessary to choose some method which would require small amounts of the material to be heated. Although the author realized that the test tube method of pasteurization is in ill repute, the decided advantages possessed by this method were responsible for its adoption for this particular study. In an effort to justify this method of procedure, extensive preliminary work was conducted in order to compare the rate of heat transfer in whole milk and in ice cream mix. The results were gratifying inasmuch as they indicated very little difference in the rate of heat transfer between these two media.

It was decided to confine the following work as much as possible to a laboratory problem since facilities for field work were not available. The work is conveniently divided into three parts as follows: the determination of strains of the colon-aerogenes group showing resistance to the pasteurizing temperatures commonly employed, the effect of the various ingredients of the ice cream mix upon the viability of the members of the colon-aerogenes group, and other factors affecting the viability of members of this group.

Determination of resistant strains.

The cultures used throughout this study were intentionally secured from various sources. The majority of the

strains were obtained from our laboratory stock cultures which had been isolated from water supplies and from the digestive tract of chickens. A list of the cultures is given in Table I together with their source when possible.

As it was desirable to use a liquid culture for inoculation purposes, the synthetic medium developed by Dolloff (14) was adopted. By the use of a synthetic medium variations in growth due to changes in constitution of a natural medium are largely eliminated.

Dolloff's medium contains the following:

Lactose	5 gms.
Ammonium tartrate	5 gms.
Ammonium phosphate	0.02
Distilled water	1000 cc.

Five cubic centimeter portions of the above medium were distributed into test tubes and sterilized in the autoclav at fifteen pounds pressure for twenty minutes.

The determination of living cells in cloudy medium presents numerous difficulties. As direct plating proved unsatisfactory for this purpose, it was necessary to resort to some other method. The use of lactose broth fermentation tubes gave the desired results and so were adopted for this work. Standard extract broth was adjusted so that the pH ranged from 6.9 to 7.0. One per cent lactose and one per cent Andrade's indicator were added. Five cubic centimeters of the medium was distributed into Durham fermentation tubes and autoclaved at fifteen pounds pressure for twenty minutes. The production of acid and gas in this medium when inoculated

with 0.1 cc. of the inoculum and incubated at 37° C. for 48 hours was assumed to be indicative of the survival of Escherichia coli.

The first portion of the work was concerned with the ice cream mix. This material was obtained directly from the holding tank of the College Dairy. The mix is pasteurized at a temperature of 145° F. (62.8° C.) for thirty minutes. The material was first distributed in exactly 10 cc. amounts in Pyrex test tubes and then heated for fifteen minutes in flowing steam on two successive days. The medium thus prepared was tested for sterility by seeding a lactose broth fermentation tube with 0.1 cc.

The cultures to be studied were grown in Dolloff's medium for twenty-four hours at 37° C. The sterile ice cream mix was inoculated with 0.1 cc. of this liquid culture. This amount contained from 10,000 to 50,000 bacteria as determined by experimental plating at the outset of the study. Care was taken thoroughly to agitate the seeded tubes to insure even distribution of the inoculum. All tubes were then incubated for two hours at 37° C. At the end of this time lactose broth fermentation tubes inoculated with one loop-full (4mm.) of the material always showed acid and gas production after being incubated at 37° C. for twenty-four hours.

All heating operations were carried out in a De Khotinsky type electrically heated and controlled water bath. This apparatus permitted the accurate regulation of the bath temperature to within 0.1° C. After adjusting the bath to the desired temperature, the tubes were immersed to within one-

half inch of their tops. A tube of ice cream mix containing a thermometer was placed on the opposite side of the tank from the bath thermometer. The 30 minute period of heating was not recorded until the temperature of the material in the tubes had reached the temperature of the water bath.

Immediately after the completion of the heating period the tubes were cooled by immersion in cold water. Lactose broth fermentation tubes were seeded with 0.1 cc. of the heated mix and incubated at 37° C. Results were recorded after twenty-four hours and forty-eight hour incubation periods.

It was soon found that the cultures showed extreme variation in their viability as affected by the heating process. Repeated trials using the same culture gave different results. These variations are possibly due to such factors as, slight change in composition and pH of the mix, variations in the numbers of bacteria seeded, and the personal error of technic. However, by a number of trials the relative viability of a particular culture could be established. In interpreting the following data it is assumed that any fermentation tube showing at least 5 per cent gas production and sufficient acid to change the color of Andrade's indicator, is indicative of the survival of that strain at the temperature heated.

The first temperature selected was 140° F. (60° C.). The difference in the effect on the viability of the colon-aerogenes group when heated in ice cream mix and in skim milk was very marked. At this temperature it will be noticed from Table 2 that of the cultures heated in the ice cream mix 23 strains or 52 per cent survived. The same cultures heated in

skim milk show a survival of 18 strains or 40 per cent. The heating was then repeated at a temperature of 145° F. (62.8°) The increased temperature resulted in a marked decrease in the viability of the cultures as it is shown in Table 3. In this case, of the cultures heated in ice cream mix only 10 strains or 22 per cent survived. The same cultures heated in skim milk under identical conditions were all destroyed. By using a higher temperature of 150° F (65.5° C,) the results given in Table 4 were obtained. The cultures were heated in ice cream mix and only four, or less than 1 per cent, survived at this temperature.

The temperature was again raised, this time to 155° F. (68.3° C). The data given in Table 5 show no cultures producing acid and gas in lactose broth. A temperature higher than 155° F. (68.3° C.) was impossible due to the fact that ice cream mix was altered in its physical state.

The above data show that ice cream mix has a greater protective effect than shown by skim milk, or, perhaps, an added effect. The question naturally arises as to what factor or factors cause this protective action. In an attempt to learn more of this protective action, each of the constituents of the ice cream mix were studied separately to determine their effect.

Effect of the Ingredients of the Ice Cream Mix Upon the Viability of Members of the Colon-aerogenes Group.

After having demonstrated that the ice cream mix, as a whole, exerts a certain protective influence upon members of

the colon-aerogenes group, attention was turned to the substances which go to make up the ice cream mix. In general, this product consists of a source of fat such as cream, a source of solids not fat such as fresh skim milk or skim milk powder, and various sweetening and stabilizing agents of which sucrose and gelatin are the most commonly used. In working with these various ingredients an attempt was made to study these materials in the same concentration in which they normally occur in the ice cream mix. As the formulae used by the ice cream manufacturer are varied according to the demands of the consumer, each ingredient was made up in concentrations which would include those used in the various formulae available.

Fat, being the most important of the constituents, was considered first. Suspensions were made up as follows: Cream testing 40 per cent butterfat was used as a base. In order to avoid changing the solids not fat content, weighed amounts of cream were added to weighed amounts of water. The resulting mixtures contained 8 per cent, 10 per cent, 12 per cent, 14 per cent, and 16 per cent respectively of butter-fat by weight. This material was distributed in 10 cc. amounts in Pyrex test tubes and sterilized by autoclaving for fifteen minutes at a pressure of ten pounds. Sterility was checked by seeding lactose broth with 0.1 cc. portions.

The inoculation, incubation and heating procedures used for fat were identical to those used for the ice cream mix. In this case, however, only those cultures were used which possessed some resistance when heated in the ice cream mix. In Table 6 are presented the results of heating certain

cultures in the presence of various percentages of fat at a temperature of 140° F. (60° C.) for thirty minutes. It will be noted that this temperature was sufficient to destroy all of the cultures used.

Skim milk powder is frequently used to supply solids not fat in ice cream mix. Water suspensions of skim milk powder were prepared in 1 per cent, 2 per cent, and 4 per cent concentrations. Sterilization was accomplished by successive heatings in flowing steam. The nine cultures used had previously survived a temperature of 140° F. (60° C.) when heated in skim milk. The results are given in Table 7 and show that when the cultures were heated in skim milk powder at a temperature of 140° F. (60° C.) for thirty minutes, four or 44 per cent of the strains survived. It is interesting to note that the strains surviving when heated in the milk powder are the same ones which survived when heated in skim milk. The same cultures were heated under identical conditions except that a temperature of 145° F. (62.8° C.) was used. All of the cultures were destroyed at this temperature.

In order to study the sucrose present in ice cream mix, water solutions of 10 per cent, 12 per cent, and 14 per cent were tubed in 10 cc. amounts. The material was sterilized by autoclaving for fifteen minutes at a pressure of ten pounds. In Table 8 are given the results obtained by heating various cultures of the colon-aerogenes group in sucrose solutions at a temperature of 140° F. (60° C.) for thirty minutes. Three or 33 per cent of the cultures survived this temperature. The temperature was then raised to 145° F. (62.8° C.). All of the cultures were destroyed.

For determining the effect of gelatin, a 0.5 per cent solution was adopted inasmuch as this concentration is quite generally used. The solution was distributed in 10 cc. amounts and autoclaved for fifteen minutes at a pressure of ten pounds. It was found that when nine cultures of the colon-aerogenes group were heated in the gelatin solution at a temperature of 140° F. (60° C.) not a single culture survived. The data thus obtained would thus tend to indicate that gelatin at this concentration has very little or no protective effect.

The Effect of Age on Viability of Members of the Colon-aerogenes Group.

In order to learn something of the effect of the age of culture used upon the viability of the organism the following experiment was conducted: In order to adopt a temperature at which the organisms would normally survive when heated in ice cream mix, it was necessary to determine the critical temperature of the strains selected. The eight cultures used showed a greater tendency to resist normal pasteurizing temperatures than the others. Tubes containing sterile ice cream mix were inoculated with 0.1 cc. of the 24 hour culture of these strains. After 2 hours incubation at 37° C. the tubes were heated at 143°, 144°, 145°, 146°, and 147° F. for a period of thirty minutes. The data presented in Table 9 are the results of lactose broth fermentation of the heated mix. It will be noted that the majority thermal death point of the cultures when heated in ice cream mix is 145° F. (63° C.).

Three sets of tubes of sterile ice cream mix were prepared. Dolloff's medium was inoculated with the above cultures and incubated at 37° C. After a six hour incubation period the first set of ice cream mix tubes was inoculated with 0.1 cc. of the liquid culture. After two hours at 37° C. the inoculated tubes of mix were heated at temperature of 145° F. (61.6° C.) for thirty minutes. The second set of tubes was inoculated when the cultures were twelve hours old and the third set after twenty-four hours incubation at 37° C. The heating temperature of 145° F. was selected from the data given in Table 9. It is quite evident as indicated in Table 10 that the age of the culture had a decided effect upon the viability of the organism.

TABLE 1

Classification and Source of Cultures

No.	NAME	SOURCE	No.	NAME	SOURCE
2	A. aerogenes	Water	55	E. coli	Avian
3	?	Water	56	E. coli	Avian
7	E. coli	Water	57	E. coli	Avian
14	A. aerogenes	Water	58	E. coli	Avian
18	E. coli	Water	59	E. coli	Avian
19	E. coli	Water	60	E. coli	Avian
29	A. aerogenes	U of I lab	100	E. coli	Water
30	E. coli	U of C lab	101	E. coli	Water
31	A. aerogenes	U of C lab	103	E. coli	Water
34	?	Water	104	E. coli	Bovine
35	?	Water	105	E. coli	Bovine
37	?	Water	107	E. coli	Avian
38	E. coli	Water	108	E. coli	Equine
39	E. coli	Water	109	E. coli	River
40	E. coli	Water	110	E. coli	Monkey
45	E. coli	Water	112	E. coli	Human
46	A. aerogenes	Water	113	E.coli	Human
47	E. coli	Water	114	E. coli	Swis
49	E. coli	Avian	116	E. coli	Bovine
50	E. coli	Avian	120	A. aerogenes	Soil
51	E. coli	Avian	121	A. aerogenes	Soil
53	E. coli	Avian			
54	E. coli	Avian			

TABLE 2

The Effect on Viability of Heating Members of the Colon-aerogenes Group in Ice Cream Mix and in Skim Milk at a Temperature of 140° F. (60° C.) for Thirty Minutes.

Strain No.	Ice Cream		Skim Milk		Strain No.	Ice Cream		Skim Milk	
	Acid	Gas	Acid	Gas		Acid	Gas	Acid	Gas
2	+	+	-	-	54	+	+	+	+
3	+	-	-	-	55	+	-	+	+
7	+	-	-	-	56	+	+	+	+
14	+	-	-	-	57	+	-	+	-
18	+	+	+	+	58	+	+	+	+
19	+	+	-	-	59	+	+	+	+
29	+	-	-	-	60	+	+	+	+
30	+	-	-	-	100	+	+	+	+
31	+	-	-	-	101	+	+	+	+
34	+	-	-	-	102	+	+	+	-
35	+	-	-	-	103	+	+	+	+
37	+	-	-	-	107	+	-	-	-
38	+	+	+	+	108	+	+	+	+
39	+	+	-	-	109	+	-	+	-
40	+	-	-	-	110	+	-	-	-
45	+	+	-	-	112	-	-	-	-
46	+	+	-	-	113	+	+	+	+
49	+	+	-	-	114	+	-	-	-
50	+	-	-	-	116	+	+	+	+
51	-	-	-	-	120	+	+	+	+
53	+	+	+	+	121	+	-	-	-

TABLE 3

The Effect on Viability of Heating Members of the Colon-aerogenes Group in Ice Cream Mix and in Skim Milk at a Temperature of 145° F. (62.8° C.) for Thirty Minutes.

Strain No.	Ice Cream		Skim Milk		Strain No.	Ice Cream		Skim Milk	
	Acid	Gas	Acid	Gas		Acid	Gas	Acid	Gas
2	+	-	-	-	54	-	-	-	-
3	-	-	-	-	55	+	-	-	-
7	+	-	-	-	56	-	-	-	-
14	+	-	-	-	57	-	-	-	-
18	+	+	-	-	58	+	-	-	-
19	+	-	-	-	59	-	-	-	-
29	-	-	-	-	60	+	+	-	-
30	-	-	-	-	100	-	-	-	-
31	-	-	-	-	101	-	-	-	-
34	-	-	-	-	102	-	-	-	-
35	-	-	-	-	103	+	+	-	-
37	-	-	-	-	107	+	+	-	-
38	+	+	-	-	108	-	-	-	-
39	+	+	-	-	109	-	-	-	-
40	+	+	-	-	110	-	-	-	-
45	-	-	-	-	112	-	-	-	-
46	-	-	-	-	113	+	+	-	-
49	+	+	-	-	114	-	-	-	-
50	-	-	-	-	116	-	-	-	-
51	-	-	-	-	120	-	-	-	-
53	-	-	-	-	121	-	-	-	-

TABLE 5

The Effect on Colon-aerogenes Cultures When Heated in Ice Cream Mix at a Temperature of 155° F. (68.3° C.) for Thirty Minutes.

No.	Acid	Gas	No.	Acid	Gas	No.	Acid	Gas
2	–	–	40	–	–	100	–	–
3	–	–	45	–	–	101	–	–
7	–	–	46	–	–	102	–	–
14	–	–	49	+	–	106	–	–
18	–	–	50	–	–	107	–	–
19	–	–	51	–	–	108	–	–
29	–	–	53	–	–	109	–	–
30	–	–	54	–	–	110	–	–
31	–	–	55	–	–	112	–	–
34	–	–	56	–	–	113	–	–
35	–	–	57	–	–	114	–	–
37	–	–	58	+	–	116	–	–
38	–	–	59	–	–	120	–	–
39	–	–	60	–	–	121	–	–

TABLE 6

The Effect on the Viability of the Colon-aerogenes Group When Heated in Various Percentages of Fat at a Temperature of 140° F. (60° C.) for Thirty Minutes.

	8%		10%		12%		14%		16%	
No.	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas
18	–	–	–	–	–	–	–	–	–	–
19	–	–	–	–	–	–	–	–	–	–
38	–	–	–	–	–	–	–	–	–	–
39	–	–	–	–	–	–	–	–	–	–
40	–	–	–	–	–	–	–	–	–	–
60	–	–	–	–	–	–	–	–	–	–
101	–	–	–	–	–	–	–	–	–	–
103	–	–	–	–	–	–	–	–	–	–

Table 7.

The Effect on the Viability of Members of the Colon-aerogenes Group When Heated in Various Water Suspensions of Skim Milk Powder at a Temperature of 140° F. (60° C.) for Thirty Minutes.

Culture No.	[illegible]		[illegible]		[illegible]	
	Acid	Gas	Acid	Gas	Acid	Gas
50	−	−	−	−	−	−
51	−	−	−	−	−	−
53	−	−	−	−	−	−
54	−	−	−	−	−	−
55	−	−	−	−	+	+
56	+	+	−	−	−	−
57	−	−	−	−	−	−
58	+	+	−	−	−	−
59	+	+	−	−	−	−

TABLE 8.

The Effect on the Viability of Members of the Coli-aerogenes Group when heated in Various Concentrations of Sucrose at a Temperature of 140° F. (60° C.) for Thirty Minutes.

No.	10%		12%		14%	
	Acid	Gas	Acid	Gas	Acid	Gas
50	−	−	−	−	−	−
51	−	−	−	−	+	+
53	−	−	−	−	−	−
54	−	−	−	−	+	+
55	+	+	−	−	−	−
56	−	−	−	−	−	−
57	−	−	−	−	−	−
58	+	−	+	−	+	−
59	−	−	−	−	−	−

TABLE 9.

The Effect of Heating Members of the Colon-aerogenes Group in Ice Cream Mix at Various Temperatures

No.	143° F. Acid	143° F. Gas	144° F. Acid	144° F. Gas	145° F. Acid	145° F. Gas	146° F. Acid	146° F. Gas	147° F. Acid	147° F. Gas
18	+	+	+	+	+	-	+	-	-	-
19	+	+	+	+	+	+	+	-	-	-
38	+	-	+	-	+	-	+	-	+	-
39	+	-	+	+	+	-	+	-	+	-
40	+	-	+	+	+	-	+	-	+	-
60	+	-	+	-	+	-	+	-	+	-
101	+	+	+	-	+	-	+	-	+	-
103	+	-	+	+	+	-	+	-	+	-
Con	-	-	-	-	-	-	-	-	-	-

TABLE 10.

The Effect of the Age of the Culture When Members of the Colon-aerogenes Group are Heated in Ice Cream Mix at a Temperature of 148° F. (61.6° C.) for Thirty Minutes.

No.	6 hours		18 hours		24 hours	
	Acid	Gas	Acid	Gas	Acid	Gas
18	-	-	-	-	+	+
19	-	-	-	-	+	+
38	-	-	-	-	+	+
39	+	-	+	-	-	-
40	+	-	+	-	+	+
60	+	-	+	-	+	+
101	+	-	-	-	+	+
103	-	-	-	-	-	-
Con	-	-	-	-	-	-

Discussion

The data submitted has provided some interesting facts which should prove useful in interpreting the significance of members of the colon-aerogenes group in ice cream. The comparison of the effect on viability produced by skim milk and by ice cream indicated definitely that the latter exhibited a greater protective action.

The testing of the ingredients contributed very little to a solution of the problem as a whole. Of all the substances tested fat was the one from which a greater protective action was expected. The heating of the cultures in fat suspensions showed great variability, however, the organisms were readily killed when heated at a temperature of 145° F. (62.8° C.) for thirty minutes. The data shows no influence of concentration of fat upon viability.

Heating culture in skim milk powder showed no correlation between concentration and viability. The cultures surviving a temperature of 140° F. (60° C.) when heated in skim milk powder were the same cultures surviving that temperature when heated in skim milk. Also three of these cultures survived in ice cream mix at the same temperature.

Cultures heated in sucrose solutions showed variation in viability, but again no correlation was observed between this effect and concentration of the solution used.

Gelatin was the one ingredient tested which exhibited the least protective effect at the concentration used (0.5%). The lowest temperature adopted, 140° F., was sufficient to kill

all cultures heated.

After it had been found that the ingredients individually exhibited no marked effect on viability, the next step would have been to test various combinations of ingredients in an effort to find the factor or factors responsible for the protective action observed. Unfortunately, lack of time did not permit consideration of this phase of the problem.

The marked effect exhibited by age of culture was found to be in accord with previous work of this nature. Thus Robertson (15) found that young cells were more susceptible than older cells to the action of high temperatures. He concluded that the most efficient pasteurization would be obtained if the heat was applied before the bacteria had passed the accelerative stage of growth.

The variations and apparent inconsistencies were not entirely unexpected since other workers have found similar variations. Ayers and Johnson (7) studied 174 cultures of Escherichia coli and found the following variations: At a temperature of 145° F. (62.8° C.) 12 cultures survived the first trial. On retesting they found four cultures surviving the second trial, eight surviving the third trial, six surviving the fourth trial, nine surviving the fifth trial, and no cultures surviving the sixth trial. The litmus milk tube method was employed. The authors concluded that a temperature of 150° F. (65.5° C.) for thirty minutes should destroy members of the colon-aerogenes group when heated in milk. If it is common for milk to show marked variations it would not

be surprising that the ice cream mix should exhibit even greater variations.

The question of pH undoubtably is closely linked with viability. According to Cohen (16) mortality is affected by small changes in pH. This factor would be impossible to control except under laboratory conditions. The mix when stored in the holding tank slowly undergoes a decrease in pH. Thus freshly made mix was found to have a pH of 6.8, whereas after 48 hours in the holding tank the pH had often dropped 5.8. The determination of the significance of this factor would constitute a separate problem in itself.

One of the outstanding characteristics of ice cream mix is its viscosity. It seems reasonable to assume that this property might have some influence on the protective action exhibited by the mix. Extensive work by Joslyn (17) on sirups showed the relationship between viscosity and heat penetration. He found that an increase in viscosity resulted in a decrease in the rate of heat penetration and that the addition of acid reduces the retardation of heat penetration. This factor which has little significance under laboratory conditions, might be of importance in the commercial pasteurization process.

The results obtained with the individual ingredients are not entirely in accord with those found by Brown and Weiser (18). They determined thermal death points by heating lactic acid bacteria in various media. The work was summarized as follows: "The thermal death point, determined in bouillon, of some of the non-spore bearing bacteria (including Escherichia coli)isolated from pasteurized milk and cream is higher than

the pasteurization temperature (145° F. for 20 min.), while many have a thermal death point below the temperature of pasteurization. The casein and fat in milk offer some protection to bacteria that are subjected to high temperature during the death point determination." It is impossible to compare the data secured by Brown and Leiser with that shown above, since a heating period of twenty minutes was used by the former and a period of thirty minutes for the latter work.

The fact that ice cream mix does possess considerable protective action for members of the colon-aerogenes group is significant. Thus it is reasonable to assume that this protection might be accorded to other groups of bacteria. If this assumption is correct it is evident that a temperature of 145° F. (62.8° C.) cannot effectively pasteurize ice cream mix.

A recent article by Price (19) discussed the significance of Escherichia coli in ice cream. The author stated that the presence of the colon-aerogenes group in ice cream does not necessarily indicate fecal contamination. And further, "Cows and other animals are not susceptible to typhoid fever and other enteric diseases of man; therefore, Bact. coli of bovine origin are without significance as far as transmission of such diseases is concerned. The numerous recorded outbreaks of typhoid and paratyphoid transmitted by milk supplies are conclusive evidence of their contamination from human sources. ..... In common with other bacteria members of this group are more indicative of the temperature at which the milk has been kept than of original contamination."

It would appear from the above article that members of the colon-aerogenes group lose most of their significance when found in dairy products.

The presence of the colon-aerogenes group in ice cream could hardly be interpreted as indicating inefficient pasteurization inasmuch as resistant strains have been found to be common. The one other factor of interest, namely, that of contamination after pasteurization is one which should be given due consideration. Probably the most common source of contamination of this type is from the hands of operators. The possibility of this source of Escherichia coli was recognized by Winslow (20) as early as 1903. A more recent article by Buice, Schested, and Dienst (21) reported 337 tests made on 251 food handlers. They found 67.7 per cent of the total number showing lactose fermenting aerobes. By culturing with Koser's sodium citrate medium, 8.4 per cent of the total proved to be of intestinal origin. Close attention to personal hygiene would eliminate this factor as one of importance.

The data available at the present time would tend to indicate that too much significance should not be accorded the colon-aerogenes group when found in ice cream. The health officer should recognize the limitations of the test for members of this group and not place too much dependence upon it in determining the sanitary quality of dairy products.

Summary.

1. A temperature of 155° F. (68.3° C.) was found necessary to kill forty-two cultures of the colon-aerogenes group when heated in ice cream mix for thirty minutes. From a commercial standpoint a temperature of 150° F. (65.5° C.) for thirty minutes was indicated for the pasteurization of ice cream mix.

2. The same culture when heated in skim milk required a temperature of only 145° F. (62.8° C.) to effect their death.

3. The various ingredients examined, namely, gelatin, cream, skim milk powder, and sucrose failed to show marked protective action on viability. A temperature of 140° F. (60° C.) killed all cultures in gelatin while the other ingredients required a temperature of 145° F. (62.8° C.).

4. The effect of age of culture on the viability of eight strains of the colon-aerogenes group was determined. It was found that all strains were killed at a temperature of 143° F. (61.6° C.) when heated in ice cream mix using cultures six hours old. Twenty-four hour cultures heated under similar conditions showed a survival of 75 per cent.

Conclusions.

1. Members of the colon-aerogenes group survived to a greater extent when heated in ice cream mix than in skim milk, when the same cultures were heated under identical conditions.

2. The ingredients of the ice cream mix exhibited separately very little influence upon the viability of members of the colon-aerogenes group.

3. The data presented show no correlation between the concentration of the ingredients used and their effect on viability.

4. The age of culture was found to be an important factor affecting the viability of the culture heated.

5. The data indicated that, in general, a temperature of 145° F. (62.8° C.) was insufficient to destroy members of the colon-aerogenes group when heated in ice cream mix for thirty minutes.

6. According to the above data little significance should be attached to the presence of members of the colon-aerogenes group when found in ice cream.

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